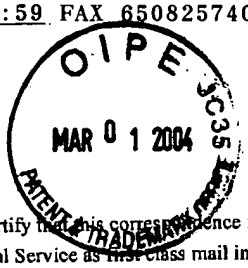

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On 25 Feb. 2004

TOWNSEND and TOWNSEND and CREW LLP

By: Malinda Dwight

PATENT

Attorney Docket No.: 018781-007410US

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No.: 2892
TIAN et al.	Examiner: Claire M. Kaufman, Ph.D.
Application No.: 09/990,940	Art Unit: 1646
Filed: November 21, 2001	Rule 1.132 Declaration
For: NOVEL RECEPTORS	
Customer No.: 20350	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

1. I, Hui Tian, Ph.D., am a scientist at Tularik Inc., a biopharmaceutical company headquartered in South San Francisco, CA and the assignee of the above-referenced patent application. I am one of the inventors of the subject matter disclosed and claimed in the above-referenced patent application.

2. I hold a Ph.D. from the University of Texas Southwestern Medical Center. I have worked in the field of molecular biology for over ten years and specifically in the field of G-protein coupled receptors for over four years. A copy of my Curriculum Vitae is attached as Exhibit A.

3. It is my understanding that the claims currently under examination, which relate to, *inter alia*, murine TGR346b nucleic acid sequences, were rejected as allegedly lacking utility. This Declaration presents exemplary data showing that TGR346 functions as a GPCR. The experiments were performed by myself or under my supervision.

4. GPCR activity can be assessed using a variety of common assays. One such assay is an Aequorin assay. Aequorin assays are widely used in the art to measure GPCR-mediated increases in intracellular calcium. The assay involves the use of the Ca^{2+}-sensitive photoprotein aequorin. The aequorin complex contains the apo-aequorin protein, molecular oxygen, and the luminophore coelenterazine. The binding of calcium ions disrupts the complex, leading to the emission of blue light, which provides a means of determining increases in intracellular calcium.

5. The peptide ligand P518 activates human TGR346. Mouse TGR346b (and mouse TGR346a) GPCR activities were tested in an Aequorin assay. Briefly, CHO cells were transiently co-transfected with 10 μg of an Aequorin reporter gene and 10 μg of a cDNA encoding mouse TGR346 or a vector control. The mouse TGR346b expression vector comprises the coding region of the cDNA sequence presented in SEQ ID NO:17, which encodes the protein of SEQ ID NO:18. Following transfection, the cells were harvested and re-suspended in buffer containing coelenterazine f. Aequorin luminescence was determined following incubation of the harvested cells with the ligand P518. The results, shown in attached Figure 1, demonstrate that mouse TGR346b has GPCR activity: it transduce an increase in intracellular calcium.

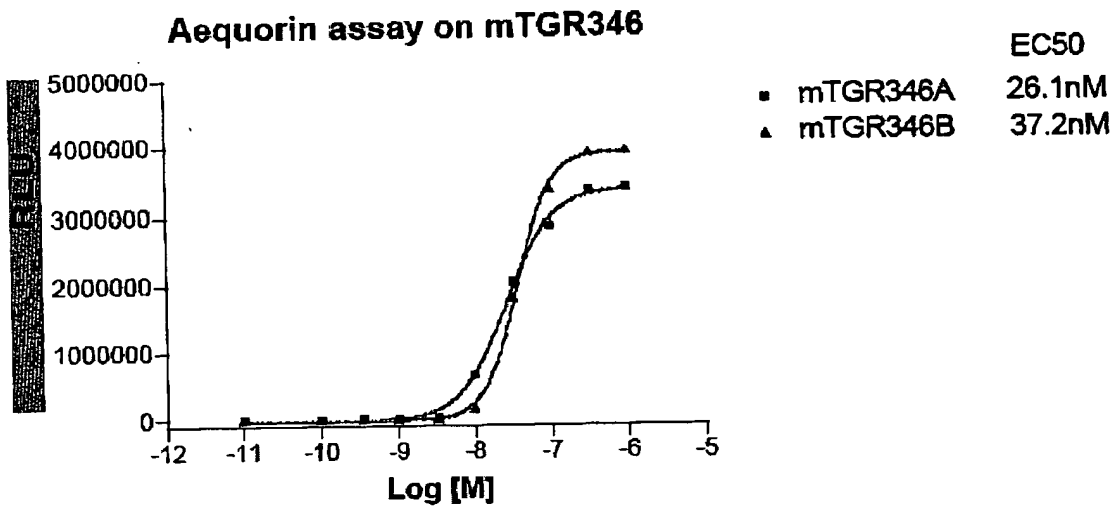
6. Screening methods for modulators of GPCRs typically entail measuring the activity of a GPCR in the presence of a test compound. The art has described that human TGR346 can be used in such screening methods to identify modulators of its activity. These modulators can be used for the treatment of various disorders such as neurological disorders (*e.g.*, WO200078809). Mouse TGR346 nucleic acid and polypeptide sequences can also be used to identify modulators both *in vitro* and *in vivo*. For example, transgenic mice for *in vivo* screening assays can be engineered using mouse TGR346 nucleic acid sequences. The modulators identified in the screening assays are used to regulate activity in cells, *e.g.*, brain cells, in whichTGR346 activity is abnormal. Thus, this invention has utility to practitioners in the art.

7. I further declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both (18 U.S.C. § 1001), and may jeopardize the validity of the patent application or any patent issuing thereon.

Date: 2/24/04 By: _____

Hui Tian, Ph.D.

Figure 1


Aequorin assay on mTGR346
RLU
5000000
4000000
3000000
2000000
1000000
0
-12
-11
-10
-9
-8
-7
-6
-5
Log [M]
EC50
mTGR346A 26.1nM
mTGR346B 37.2nM

Curriculum Vitae

Hui Tian
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Birth/Citizenship:
January 9,1968, Xian, P.R. China. Citizen of USA

Education:
University of Texas Southwestern Medical Center, Dallas
Ph.D. in Cell Regulation Program, August, 1998

Chinese Academy of Science, Shaanxi Institute of Microbiology, China
M.S. in Microbiology, July, 1992

University of Science & Technology of China
B.S. in Molecular and Cellular Biology, July, 1989

Professional experience:

6/02-present: Research Investigator, Department of Biology. GPCR target validation and assay developemnt

6/99-6/02: Scientist, Department of Drug Discovery and Biology. Supervise assay development and high throughput screening in the area of hypercholesterolemia; conduct target validation of orphan G protein coupled receptors

8/98-6/99: Postdoctoral fellow, laboratory of Dr. Andrew P. McMahon, Harvard University. Project: Dissection of the role of Wnt-4 signaling in kidney development.

6/93-8/98: Graduate student, laboratories of Drs. Steven L. McKnight and David W. Russell, University of Texas Southwestern Medical Center. Doctoral dissertation: Characterization of novel mammalian bHLH-PAS domain proteins expressed in nervous system and vascular system.

7/90-6/93: Research Associate, Chinese Academy of Science, Shaanxi Institute of Microbiology. Project: Cyclodextrin glucanotransferase from an Alkalophilic Bacillus: molecular cloning and enzyme immobilization.

9/90-12/90: Teaching assistant, Northwestern University, China.

3/89-7/89: Research assistant, University of Science and Technology of China.

Publications:
Hui Tian and Botai Xie. (1993). Molecular biology of the cyclodextrin glucanotransferase. Biochemistry of Life, 13,30-32. (Chinese).

Hui Tian, Botai Xie, Liankui Sun, Guowu Yang, and Yiling Xu. (1995). Immobilization of cyclodextrin glucanotransferase on chitosan pretreated with formaldehyde. Journal of Northwestern University, 25, 223-226. (Chinese)

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Hui Tian*, David M. Berman*, and David W. Russell. (1995). Expression and regulation of steroid 5 alpha-reductase in the urogenital tract of the fetal rat. Molecular Endocrinology, 9, 1561-1570. * These authors contributed equally

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Yu-Dong Zhou, Mary Barnard, Hui Tian, Xu Li, Huijun Z. Ring, Uta Francke, John Shelton, James Richardson, David W. Russell, and Steven L. McKnight. (1997). Molecular characterization of two mammalian bHLH-PAS domain proteins selectively expressed in the central nervous system. Proc. Natl. Acad. Sci. USA., 94, 713-718.

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Lin DC, Bullock CM, Ehlert FJ, Chen JL, Hui Tian*, Zhou QY. (2002). Identification and molecular characterization of two closely related G protein-coupled receptors activated by prokineticins/endocrine gland vascular endothelial growth factor. J Biol Chem., 277(22):19276-80. * Corresponding author

Jamila Gupte, Gene Cutler, Jin-Long Chen, Hui Tian (2004). Elucidation of signaling properties of vasopressin related receptor by using chimeric receptor approach. Proc. Natl. Acad. Sci. USA., 101, 1508-1513.